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Effect of salt concentration on indole acetic acid production by *Rhizobium* sp. nodulating horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.]

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ABSTRACT

Production of indole acetic acid by salt tolerant strains of *Rhizobium* from horse gram [*Macrotyloma uniflorum* (Lam.) Verdc] was investigated. Out of 32 strains of *Rhizobium*, eight strains were found to be efficient for IAA production at various salt concentrations. These strains produced maximum indole acetic acid at control, 0.2M and 0.4M salt concentration. *Rhizobium* strains can be exploited for IAA production under salt stress.

Key words : Salinity, Salt stress, IAA, Salt tolerant, *Rhizobium*, [*Macrotyloma uniflorum* (Lam.) Verdc].

INTRODUCTION

Tropical soils are known for their deficiency in nitrogen under high salinity, temperature and acidic stress, which demand stress tolerant microorganisms for precise agricultural development (Hungria and Vargas, 2000). Reclamation of these soils through biological system is one sort of eco- friendly and cost effective approach. The *Rhizobium*–legume sysmbiosis is suggested to be the ideal solution to the importance of soil fertility rehabilitation of arid lands and is important direct for future research. The *Rhizobium* strains tolerant to extreme conditions of soil salinity, temperature and pH can help to improve biological nitrogen fixation and thus help in soil amelioration. Stress tolerant Rhizobia, which show PGPR activities [such as production of phytohormones like indole acetic acid (IAA), siderophores and solubilization of minerals] under high salinity are desirable for further selection and improvement of the strain and other commercial applications. Application of hormones can benefit plants under salt stress (Prisco and O'Leary, 1973 and Zhao *et al.*, 1980). Though much literature is available on the role of microorganisms on IAA production, very little information is available on the effect of salt stress on IAA production by bacteria. Hence, the present study was undertaken to investigate the influence of salt concentration on the production of IAA.

MATERIALS AND METHODS

The *Rhizobium* strains were isolated from horse gram [*Macrotyloma uniflorum* (Lam.) Verdc] plants growing in thirty two soil samples collected from various parts in Andhra Pradesh. These *Rhizobium* strains were authenticated by nodulation tests. They were designated as HGR1 to HGR32. These strains were found to be highly salt tolerant. All the thirty two strains were screened for IAA production at various salt concentrations from 0 - 2M containing 0.1% L- tryptophan. The formation of indole compounds was detected by using Kovac's reagent. All the strains were positive for indole production up to 1M salt concentration. Eight strains showed IAA production even at 2M salt concentration. The rhizobial strains HGR4, HGR6, HGR8, HGR13, HGR18, HGR22, HGR23 and HGR30 which showed IAA production at 2M salt concentration were selected to study the quantitative measurement of IAA at various salt concentrations. For this, yeast extract mannitol broth containing different concentrations of sodium chloride from 0 (without salt), 0.2M, 0.4M, 0.6M, 0.8M and 1M were supplemented with 0.1% L-tryptophan. They were inoculated with 1ml of inoculum of each strain and were incubated for twenty days on gyrorotary shaker at 28±2°C. Culture broth was centrifuged at 10,000g for 15 minuites. The pelleted bacterial cells were separated by filtration and the supernatant was used for the estimation of IAA by the method described by Reddy and Reddy (2002). To find out the maximum production of IAA by these *Rhizobium* strains, IAA was determined for every 24hr up to twenty days. Uninoculated flask was kept as control.

RESULTS AND DISCUSSION

The amount of IAA produced by these strains (Table 1) was determined. The production of IAA was maximum between four to ten days except only one strain (HGR5 produced after fifteen days). Eleven strains showed high IAA production at their maximum growth. The remaining strains showed no correlation between the maximum production of IAA and their growth. The amount of IAA produced varied from 32.0 µg/ml (HGR2) to 116.0 µg/ml (HGR8).

The amount of IAA produced by these strains varied with salt concentrations and the strain involved (Fig.1).

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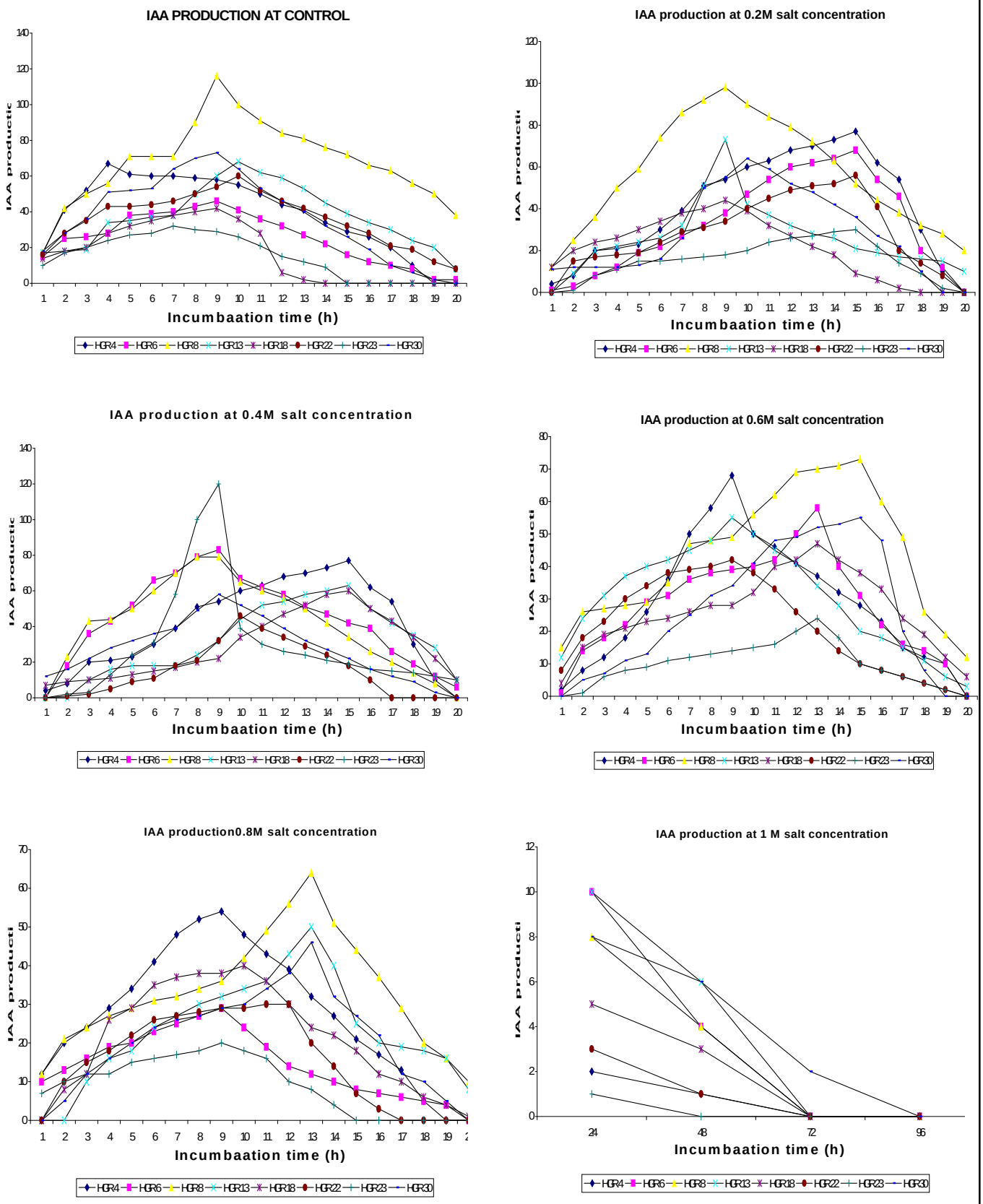

Fig. 1 : Effect of salt concentrations on IAA production by *Rhizobium* strains nodulating horse gram

In control, IAA production started after 24 hours. The amount of IAA production varied from116.0 µg/ml (HGR8) to 30.0 µg/ml (HGR23). These strains showed maximum IAA production between four to ten days. The strains HGR6, HGR8, HGR13 and HGR22 are able to produce IAA even after twenty days. In these four strains HGR8 showed very high IAA production (116.0 µg/ml) than other strains.

At 0.2M salt concentration, three strains HGR8, HGR13 and HGR18 showed maximum IAA production after nine days, while HGR4, HGR6 and HGR23 produced more IAA after fifteen days. At this salt concentration, the strain HGR8 showed very high (98.0 µg/ml) IAA production. After ten days the strain HGR30 produced more (64.0 µg/ml) IAA. The strain HGR22 produced maximum IAA (56.0 µg/ml) after fifteen days. Four strains HGR4, HGR6, HGR8 and HGR13 are able to produce IAA after twenty days (6.0 µg/ml, 20.0 µg/ml and 13.0 µg/ml).

Table 1 : Indole acetic acid production by *Rhizobium* strains nodulating Horse gram

Strain No.	Maximum growth OD at 610 nm	Growth a maximum IAA production OD at 610 nm	Time taken for maximum IAA production (in days)	IAA maximum Production (µg/ml)
HGR1	0.35	0.30	6	50.0
HGR2	0.30	0.10	4	32.0
HGR3	0.60	0.30	5	43.0
HGR4	0.50	0.50	4	67.0
HGR5	0.60	0.46	15	45.0
HGR6	0.43	0.29	9	46.0
HGR7	0.37	0.36	9	53.0
HGR8	1.20	1.02	9	116.0
HGR9	0.35	0.34	5	40.0
HGR10	0.80	0.74	9	68.0
HGR11	0.48	0.48	9	66.0
HGR12	0.32	0.25	6	36.0
HGR13	1.02	1.02	10	68.0
HGR14	0.35	0.35	9	59.0
HGR15	0.61	0.25	5	62.0
HGR16	0.51	0.42	9	58.0
HGR17	0.60	0.30	5	40.0
HGR18	0.32	0.17	9	42.0
HGR19	0.56	0.41	4	56.0
HGR20	0.32	0.12	4	56.0
HGR21	0.45	0.24	4	51.0
HGR22	0.60	0.60	10	60.0
HGR23	0.30	0.17	8	30.0
HGR24	0.43	0.32	9	50.0
HGR25	0.29	0.29	9	43.0
HGR26	0.55	0.43	6	55.0
HGR27	0.28	0.28	7	35.0
HGR28	0.47	0.47	9	40.0
HGR29	0.83	0.83	9	67.0
HGR30	0.53	0.53	9	73.0
HGR31	0.15	0.12	7	35.0
HGR32	0.64	0.64	9	70.0

At 0.4M salt concentration the strains HGR4, HGR18 and HGR30 showed IAA production after 24hr, later the production of IAA slowly increased with incubation period. Four strains HGR6, HGR8, HGR23 and HGR30 produced more IAA after nine days (83.0 µg/ml, 79.0 µg/ml, 120.0 µg/ml and 58.0 µg/ml). The strains HGR4, HGR6, HGR13, HGR18 and HGR23 are capable of producing IAA even after (6.0 µg/ml to 10.0 µg/ml) after twenty days. At this salt concentration stimulation in IAA production was observed by the strain HGR23. The amount of IAA produced by the strain HGR23 was also maximum (120.0 µg/ml) than all the strains at all the salt concentrations tested. The strains HGR13 and HGR18 showed maximum (63.0 µg/ml and 60.0 µg/ml) IAA production after fifteen days. The strain HGR22 produced more IAA after ten days (46.0 µg/ml) while the strain HGR4 showed after thirteen days (73.0 µg/ml).

At 0.6M salt concentration three strains HGR4, HGR13 and HGR22 showed maximum IAA production (68.0 µg/ml, 55.0 µg/ml and 42.0 µg/ml) after nine days. The strains HGR6, HGR18 and HGR23 showed high IAA production after thirteen days (58.0 µg/ml, 47.0 µg/ml, and 24.0 µg/ml). Two strains HGR8 and HGR30 produced more IAA (73.0 µg/ml and 55.0 µg/ml) after fifteen days. Only three strains HGR8, HGR13 and HGR8 are capable of producing IAA after twenty days.

At 0.8M salt concentration the strain HGR22 showed maximum (30.0µg/ml) IAA production after eleven and twelve days, while the strains HGR8 and HGR30 produced more IAA after thirteen days (64.0 µg/ml and 46.0 µg/ml). The amount of IAA produced was high after nine days by the strains HGR4, HGR6 and HGR23 (54.0 µg/ml, 29.0 µg/ml and 20.0 µg/ml). The strain HGR18 produced more IAA after ten days (40.0 µg/ml). Five strains *viz.*, HGR6, HGR8, HGR13, HGR18 and HGR23 able to produce IAA (2.0 µg/ml, 10.0 µg/ml, 10.0 µg/ml, 8.0 µg/ml, 1.0 µg/ml and 10.0 µg/ml) after twenty days.

At 1M salt concentration, these eight strains showed maximum IAA production (1.0 µg/ml to 10.0 µg/ml) after 24h. There is a sharp decline in IAA production was observed with incubation period. None of the strains produced IAA after four days (96h). Statistical analysis using ANOVA test showed that the variation in the amount of IAA production at different salt concentrations by

different *Rhizobium* strains is highly significant at 5% level.

From the above study, it is clear that these salt tolerant *Rhizobium* strains produced IAA even at 1M salt concentration. Three *Rhizobium* strains HGR8, HGR22 and HGR30 showed high IAA production at control. The strains HGR4 and HGR13 produced maximum IAA at 0.2M (1.1688%) salt concentration. Three strains HGR6, HGR18 and HGR23 showed maximum IAA production at 0.4M (2.3376%) salt concentration. There are few reports on IAA production under salt stress. Serpil (2002) and Yongyin *et al.* (2001) reported that the production of IAA declined in response to salinity. Beltra *et al*. (1980) observed that the production of IAA decreased after four days (96h) incubation with *Rhizobium phaseoli* and *Rhizobium leguminosarum*. In the present study the *Rhizobium* strains from horse gram are able to produce IAA even after twenty days in control and also at various salt concentrations (0.2M, 0.4M. 0.6M, 0.8M).

Since *Rhizobium* strains from horse gram can withstand high salt concentration and bring about IAA production, they can be used as bioinoculants for reclamation of saline soils.

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